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Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase

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Summary

Glutamic acid decarboxylase (GAD), the enzyme that synthesizes the neurotransmitter γ-aminobutyric acid (GABA), has been localized in the rat visual cortex by immunocytochemical methods with both light and electron microscopy. In both colchicine-injected and non-injected preparations of the visual cortex, GAD-positive reaction product was observed in somata, proximal dendrites and axon terminals of non-pyramidal neurons. The GAD-positive terminals were observed to form symmetric synaptic junctions most commonly with dendritic shafts and somata of pyramidal and stellate neurons and less frequently with initial axon segments of pyramidal neurons and dendritic spines. In colchicine-injected preparations, GAD-positive somata were located in all cortical layers including the immediately subjacent white matter. In contrast, sections from non-injected rats displayed GAD-positive somata within a superficial and a deep cortical band. The GAD-positive somata observed in both types of preparations received both symmetric and asymmetric synaptic junctions, lacked apical dendrites, and had radially oriented dendrites of small diameter. These characteristics of GAD-positive neurons indicate that they are aspinous and sparsely-spinous stellate neurons. The localization of GAD within these neurons in combination with physiological and pharmacological data indicate that these local circuit neurons mediate GABA-ergic inhibition in the neocortex.

Introduction

Glutamic acid decarboxylase (GAD), the enzyme that synthesizes the neurotransmitter γ-aminobutyric acid (GABA), has been immunocytochemically localized within specific neuronal types in the cerebellum, spinal cord, retina, Ammon's horn, olfactory bulb and substantia nigra (McLaughlin *et al.*, 1974, 1975; Saito *et al.*, 1974; Barber and Saito, 1976; Wood *et al.*, 1976; Ribak *et al.*, 1976, 1977, 1978). In all of these brain regions, GAD-positive reaction product was observed within neurons which have been classified as GABA-ergic neurons on the

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basis of findings from various biochemical, physiological and pharmacological studies. Therefore, these findings indicate that the presence of GAD is diagnostic for those neurons which use GABA as a neurotransmitter.

In the present study, GAD immunocytochemistry has been used to identify GABA-ergic neurons in the rat visual cortex. An immunocytochemical localization of GAD was undertaken in the cerebral cortex because this brain region has been studied extensively with both light and electron microscopic methods. Ramón y Cajal (1911) and Lorente de Nó (1938) have described two basic types of Golgi-impregnated neurons in the cerebral cortex: the pyramidal and the non-pyramidal (stellate) neurons. The pyramidal neuron is characterized by a spine-bearing, single, apical dendrite that ascends toward the pial surface, and by many spine-bearing, basal dendrites which radiate laterally from the cell body. These neurons are located in neocortical layers II–VI and are responsible for corpus callosal, corticocortical, corticothalamic and corticomesencephalic projections (Jacobson and Trojanowski, 1974, 1975; Lund *et al.*, 1975). In contrast, non-pyramidal or stellate neurons do not appear to be as morphologically homogeneous as the pyramidal neurons. For example, stellate neurons are characterized by either bipolar or multipolar dendrites which may not bear spines. Both types of stellate neurons, spinous and aspinous, are located in all of the six cortical layers and have short axons with intracortical connections (Ramón y Cajal, 1911; Lorente de Nó, 1938; Marin-Padilla, 1969; LeVay, 1973; Lund, 1973; Szentágothai, 1973; Jones, 1975; Valverde, 1976; Feldman and Peters, 1978). Because stellate neurons are of particular importance to this study, more details of their morphology and synaptic connections will be given in the Discussion.

The results of a number of different studies indicate that GABA is an inhibitory neurotransmitter in the rat visual cortex. Biochemical analyses show that both GAD and GABA are present in low amounts throughout the rat cerebral cortex (Okada *et al.*, 1971; Tappaz *et al.*, 1976), and that somewhat higher concentrations of GABA occur in the more superficial cortical layers (Hirsch and Robins, 1962). Following stimulation of the geniculocortical pathway in the cat, an excitatory response is recorded in the visual cortex, which is followed by an inhibitory response (Watanabe *et al.*, 1966; Armstrong, 1968). This inhibition is always delayed relative to the excitatory response, and it is thought to arise from the intracortical connections of stellate neurons. This inhibitory effect of stellate neurons in the visual cortex may be mediated by GABA since geniculocortical stimulation causes an increase of GABA release above the unstimulated background levels (Iversen *et al.*, 1971), and iontophoresis of GABA in area 17 of the cat has a strong inhibitory action on all cortical units tested (Dreifuss *et al.*, 1969; Wallingford *et al.*, 1973). Furthermore, when bicuculline, a GABA antagonist, is iontophoresed into cat visual cortex, an increased evoked and spontaneous activity of single units occurs which is probably due to a blockade of GABA-mediated inhibition (Sillito, 1975). Thus, these studies in combination with neuroanatomical investigations of the cerebral cortex suggest that some stellate neurons may use GABA as an inhibitory neurotransmitter in the visual

cortex. The main goal of the present investigation was to test this suggestion by using an immunocytochemical localization of GAD to identify the GABA-ergic neurons of the visual cortex.

Materials and methods

Sixteen, adult Sprague–Dawley rats were used in this study. The visual cortices of eight rats were injected with 2–5 μl of a colchicine solution (10 μg/μl saline, Sigma Chemical Co.). The injections of this solution were administered from a 10 μl syringe that was mounted on a micromanipulator, and the heads of the rats were held stationary during the injections by a stereotaxic apparatus. Following a 24 h survival time, the colchicine-injected rats were perfused with the same perfusion technique that was used on non-injected rats (see below). Colchicine was injected into the visual cortex in order to interrupt axonal transport and thereby produce detectable GAD concentrations within the somata and dendrites of neurons which normally show GAD localized to only their axon terminals (Ribak *et al.*, 1978). Therefore, sections used for GAD immunocytochemistry were selected primarily from colchicine-injected specimens so that GAD-containing neurons could be more completely characterized.

All rats were anaesthetized by intraperitoneal injections of 35% chloral hydrate and were then fixed via intracardiac perfusions with solutions containing 4% paraformaldehyde, 0.4% glutaraldehyde and 0.002% $CaCl_2$ in 0.12 M Millonig's (1961) phosphate buffer at pH 7.2 and 37° C. Brains were dissected from the crania the following day and specimens of visual cortex were obtained from both colchicine-injected and non-injected rats. In the case of colchicine-injected rats, specimens were selected close to injection sites which were identified by the presence of small blood clots. Specimens of visual cortex selected for light microscopy were immersed overnight in a cryoprotectant 30% sucrose solution, rapidly frozen and sectioned on a cryostat perpendicular to the pial surface at a thickness of 40 μm. After rinsing in buffer overnight, selected sections were processed for GAD immunocytochemistry as described previously (Ribak *et al.*, 1976). In addition to the routine light microscopic examination of sections of the visual cortex, neurons containing GAD-positive reaction product within their somata were drawn at 100 x magnification using a light optical microscope equipped with a drawing tube. Drawings of sections from both colchicine- and non-injected specimens were used for a comparative laminar analysis of GAD-positive somata in area 17.

For electron microscopy, unfrozen sections of the visual cortex were sectioned perpendicular to the pial surface at a thickness of 150 μm using a Sorvall TC-2 tissue sectioner. Specimens were placed in phosphate buffer, processed for electron microscopic GAD immunocytochemistry, embedded in Epon–Araldite resin and examined with an electron microscope as described previously (Ribak *et al.*, 1976). In addition, several series of 50–100 ultrathin sections were examined for a more thorough characterization of the somata and dendrites of GAD-positive neurons.

Results

Light microscopic localization of GAD

Light microscopic preparations of area 17 of the rat visual cortex (as defined in Ribak and Peters, 1975) which were incubated in anti-GAD serum showed specific reaction product for GAD throughout all cortical layers including the immediately subjacent white matter. GAD-positive reaction product appeared within somata and dendrites of neurons and within punctate structures previously identified as

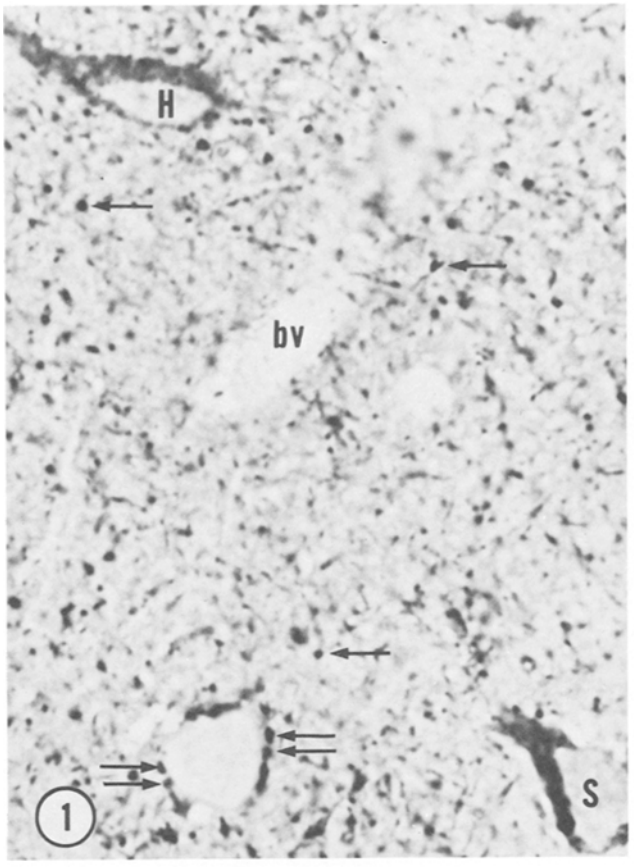
axon terminals (McLaughlin *et al.*, 1974; Ribak *et al.*, 1976; Wood *et al.*, 1976). Many GAD-positive axon terminals were distributed homogeneously within the neuropil between neuronal somata, and some terminals were located upon the surfaces of almost every soma observed in the visual cortex (see Figs. 1–3). GAD-positive terminals adjacent to the pyramidal neurons of layers III and V were so numerous that they essentially formed a continuous sheet around these neurons (see Fig. 3). This pattern of GAD-positive terminals around pyramidal neurons was similar to that formed by *boutons terminaux* and *en passant* of the pyramidal, pericellular plexus which arise from short-axon stellate neurons (Ramón y Cajal, 1911; Marin-Padilla, 1969; Shkol'nik-Yarros, 1971). A similar distribution of GAD-positive axon terminals was also observed in other cortical areas of the rat cerebrum—motor, somatosensory, and auditory (Ribak, unpublished observations).

The GAD-positive somata observed in the visual cortex were rather homogeneously distributed throughout all of the cortical layers (Figs. 1–3 and 8). Large and small GAD-positive somata displayed either round or fusiform shapes. Fusiform, GAD-positive somata usually exhibited staining that extended into the bipolar dendrites which arose from the tapered ends of each soma. The nuclei of these neurons exhibited an ellipsoidal shape and lacked reaction product. The fusiform, GAD-positive somata which had their long axes parallel to the pial surface were classified as horizontal neurons (Figs. 1 and 5), and they were present within layers I and VI. Other fusiform somata in layers III–VI were usually oriented with their long axes perpendicular to the pial surface (Figs. 2 and 6). Round, GAD-positive somata, in contrast to the fusiform somata, had round nuclei (Fig. 3) and displayed

Figs. 1–3. Semithin, 2 μm sections of colchicine-injected visual cortex incubated in anti-GAD serum. Fig. 1 shows the GAD-positive somata of a horizontal cell (H) in lower layer I and a round stellate cell (S) in upper layer II. Another soma in layer II lacks reaction product but has many GAD-positive axon terminals (double arrows) upon its surface. Numerous other GAD-positive terminals (single arrows) and a blood vessel (bv) are contained within this field. x 1300. Fig. 2 is from layer III and shows a GAD-positive fusiform soma (F) and several other unstained somata. x 1300. Fig. 3 shows a pyramidal soma (P) in layer V with a part of its pericellular plexus formed by GAD-positive axon terminals (arrows). Two GAD-positive somata (S) also have GAD-positive terminals upon their surfaces. x 1300.

Fig. 4. Semithin section through layer III of colchicine-injected visual cortex that was incubated in control serum. A cluster of apical dendrites and a blood vessel (bv) are present in the centre of the micrograph. The somata and neuropil structures lack reaction product. x 1300.

Figs. 5–7. Layer VI, GAD-positive somata in 40 μm frozen sections of colchicine-injected visual cortex incubated in anti-GAD serum. Fig. 5 shows a horizontal cell with dendrites arising from the tapered ends of its soma. One of the bipolar dendrites bifurcates a short distance from the soma (arrow). x 1200. Fig. 6 shows a fusiform, GAD-positive soma with a horizontal dendrite which sends a branch (arrow) towards the pial surface. The other GAD-positive soma in the figure is round and shows a dendrite directed towards the white matter (arrowhead). x 1200. Fig. 7 shows a GAD-positive soma with radially oriented dendrites of small diameter (arrows). In Figs. 5–7, GAD-positive axon terminals are scattered rather homogeneously throughout the neuropil. x 1200.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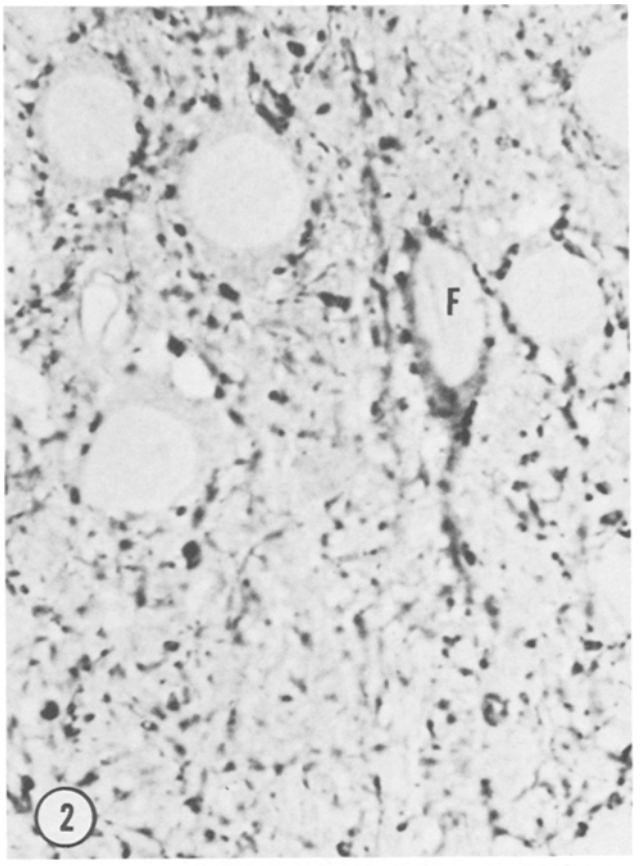
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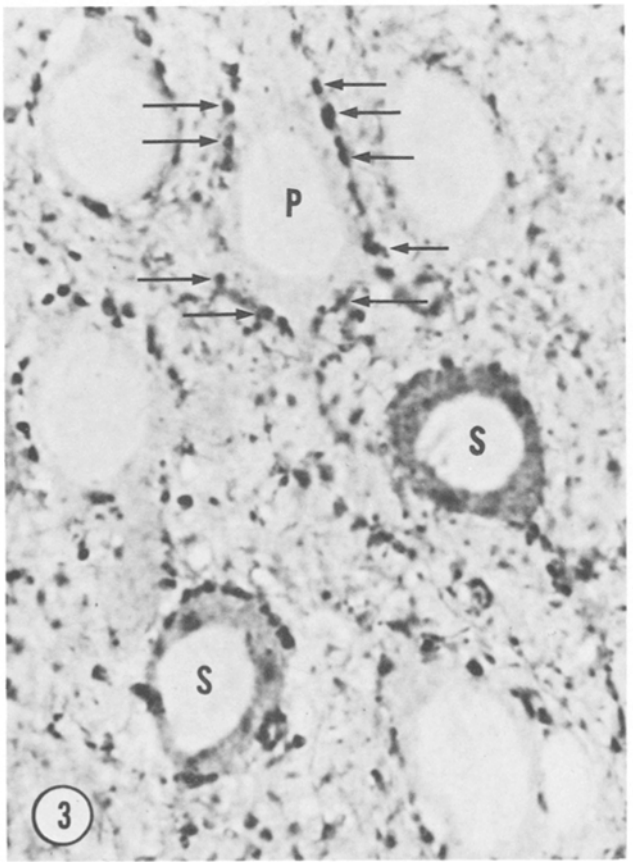
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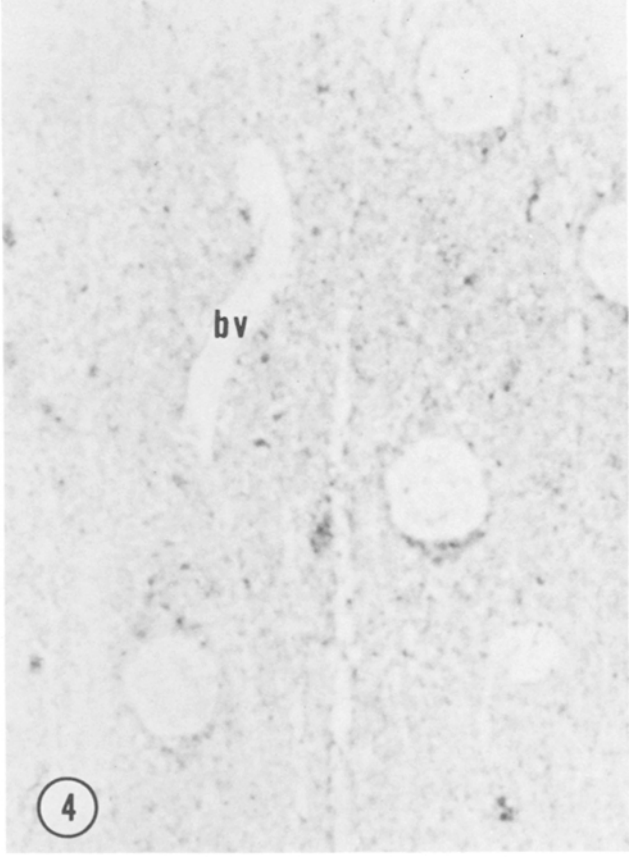
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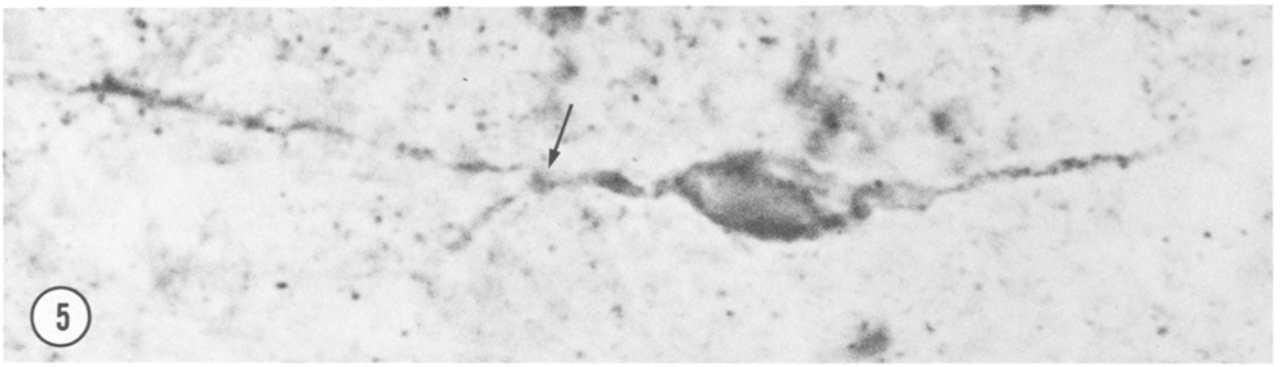

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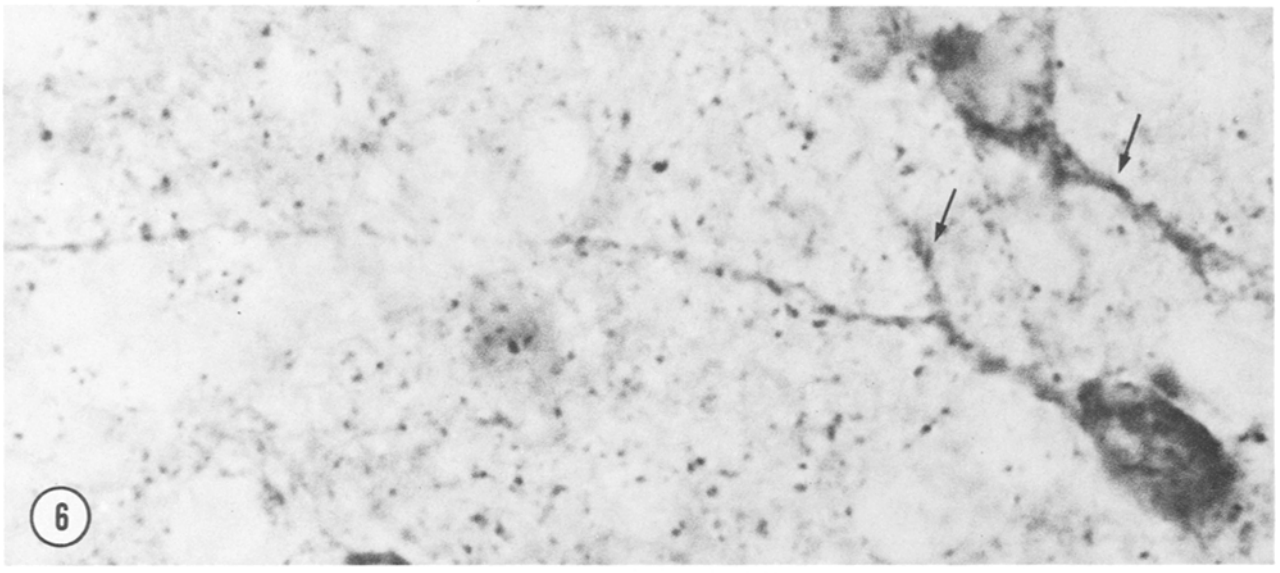

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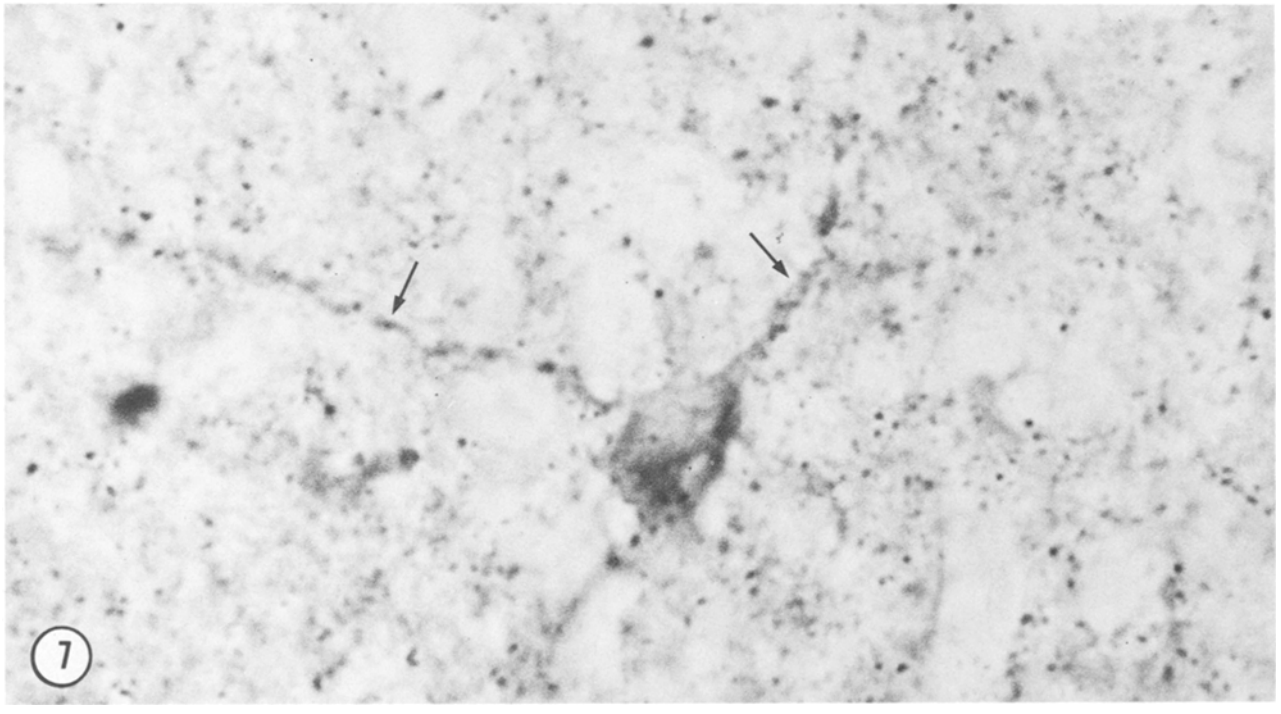

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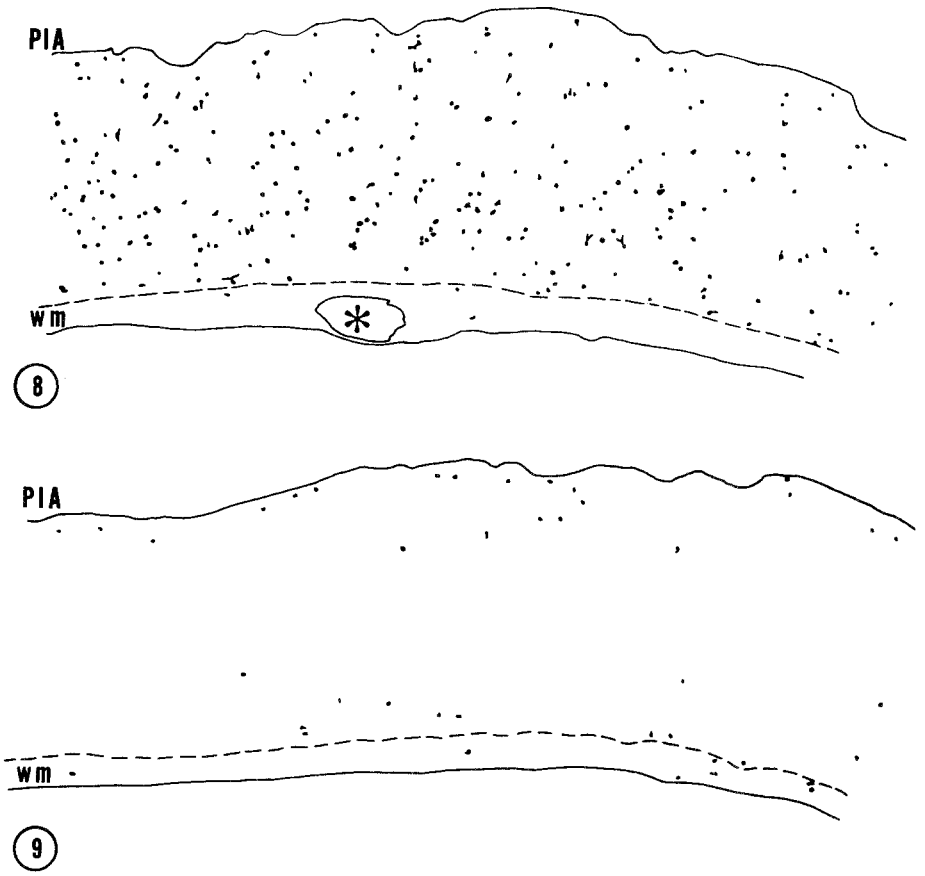
multipolar dendrites which radiated from the perikarya in all directions (Figs. 6 and 7). These multipolar dendrites were smaller in diameter than the large, unstained apical dendrites of GAD-negative pyramidal neurons (Fig. 3). Control sections of visual cortex incubated in non-immune rabbit serum did not exhibit specific reaction product (Fig. 4).

Sections of visual cortex from non-injected specimens treated with anti-GAD serum displayed a distribution of GAD-positive axon terminals similar to that observed in the visual cortex of colchicine-injected specimens. However, the distribution and numbers of GAD-positive somata differed greatly between these two preparations. Sections of colchicine-injected visual cortex exhibited about eight times more GAD-positive somata than those observed in the non-injected preparations (Figs. 8 and 9). In contrast to the distribution of GAD-positive somata



Figs. 8 and 9. Drawings of GAD-positive somata from 40 μm frozen sections of rat visual cortex incubated in anti-GAD serum. Fig. 8 is a colchicine-injected preparation and shows GAD-positive somata and dendrites throughout all cortical layers including a few somata in the white matter (wm). Damage to the tissue from the injection is indicated by an asterisk (*). x 35. Fig. 9 shows two bands of GAD-positive somata present in non-injected preparation; a superficial band in layers I and II and a deep band in layer VI and the white matter (wm). x 26.

from injected specimens (Fig. 8), GAD-positive somata in non-injected specimens were predominantly located within two laminar bands; a superficial band in layers I and II, and a deep band in layer VI and the underlying white matter (Fig. 9). The GAD-positive somata in the non-injected specimens were more difficult to classify into neuronal types because they exhibited significantly less dendritic staining than neurons observed in colchicine-injected preparations. Nevertheless, the general shapes and sizes of GAD-positive somata in non-injected specimens suggested that they belonged to the same neuronal types as those in colchicine-injected specimens.

Electron microscopic localization of GAD

Electron microscopic preparations of colchicine-injected visual cortex incubated in anti-GAD serum displayed GAD-positive reaction product within certain somata, dendrites and axon terminals throughout all cortical layers. This distribution of reaction product was consistent with the light microscopic observations presented

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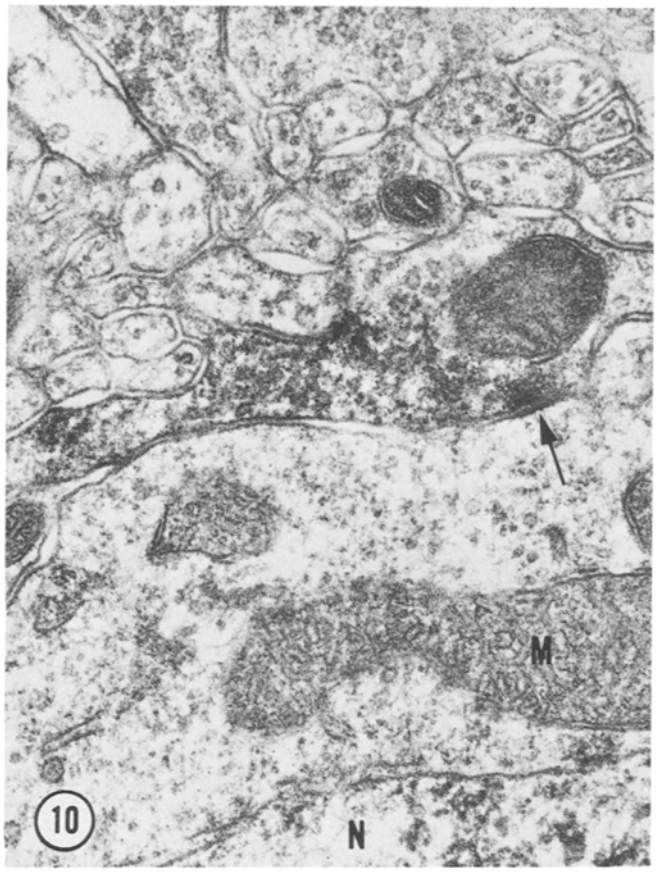
Figs. 10–12. Electron micrographs of visual cortex incubated in anti-GAD serum. Fig. 10 shows a GAD-positive *bouton en passant* which forms a symmetric synapse (arrow) with a soma in layer III. Electron-opaque reaction product appears adjacent to synaptic vesicles. The soma contains a prominent mitochondrion (M), and part of the nucleus (N). x 53 000. Fig. 11 shows two GAD-positive axon terminals which form symmetric synapses (arrows) with a portion of the soma of a layer V pyramidal neuron. One of these terminals also forms a symmetric synapse (arrowhead) with a dendrite (d). x 38 000. In Fig. 12, many GAD-positive axon terminals form symmetric synapses (arrows) with the proximal shaft of an apical dendrite (AP) of a layer V pyramidal neuron. A punctum adhaerens (arrowhead) is associated with one of these synapses. An axon terminal which lacks reaction product (AT) forms an asymmetric synapse with a spine. x 16 000.

Fig. 13. GAD-positive axon terminal forms a symmetric synapse (arrow) with an initial axonal segment (AX) of a layer II pyramidal neuron. The axon arises from the hillock region on the left and displays fasciculation of microtubules (m), a cisternal organelle (c) and dense subaxolemmal undercoating (D). x 25 000.

Fig. 14. A GAD-positive soma from a non-injected specimen is shown. The nucleus is infolded and displays a nucleolus (Nu) and an intranuclear rod (IR). Electron-opaque GAD-positive reaction product is concentrated in the cytoplasm around the cisternae of the Golgi complex (G). The cisternae of granular endoplasmic reticulum (ER) aggregate at one pole to form a Nissl body that lacks reaction product. This soma forms a punctum adhaerens (arrow) with an adjacent soma. x 14 000.

Figs. 15 and 16. Electron micrographs of a GAD-positive stellate soma from layer IV of a non-injected preparation. Fig. 15 is a higher magnification of a portion of this soma (indicated by the box in Fig. 16) which shows an example of the two types of axo-somatic synaptic junction located on GAD-positive somata. One axon terminal (GT) contains GAD-positive reaction product and forms a symmetric synaptic junction (arrow). The other axon terminal (AT) lacks reaction product and forms an asymmetric synaptic junction (arrowhead). x 46 000. In Fig. 16, this large soma is shown to contain an infolded nucleus (N), scattered cisternae of granular endoplasmic reticulum (ER), and cisternae of Golgi complex (G) that have GAD-positive reaction product concentrated around them. In addition to the synaptic junctions shown in Fig. 15, this soma forms asymmetric synaptic junctions (arrows) with axon terminals which lack reaction product. x 15 000.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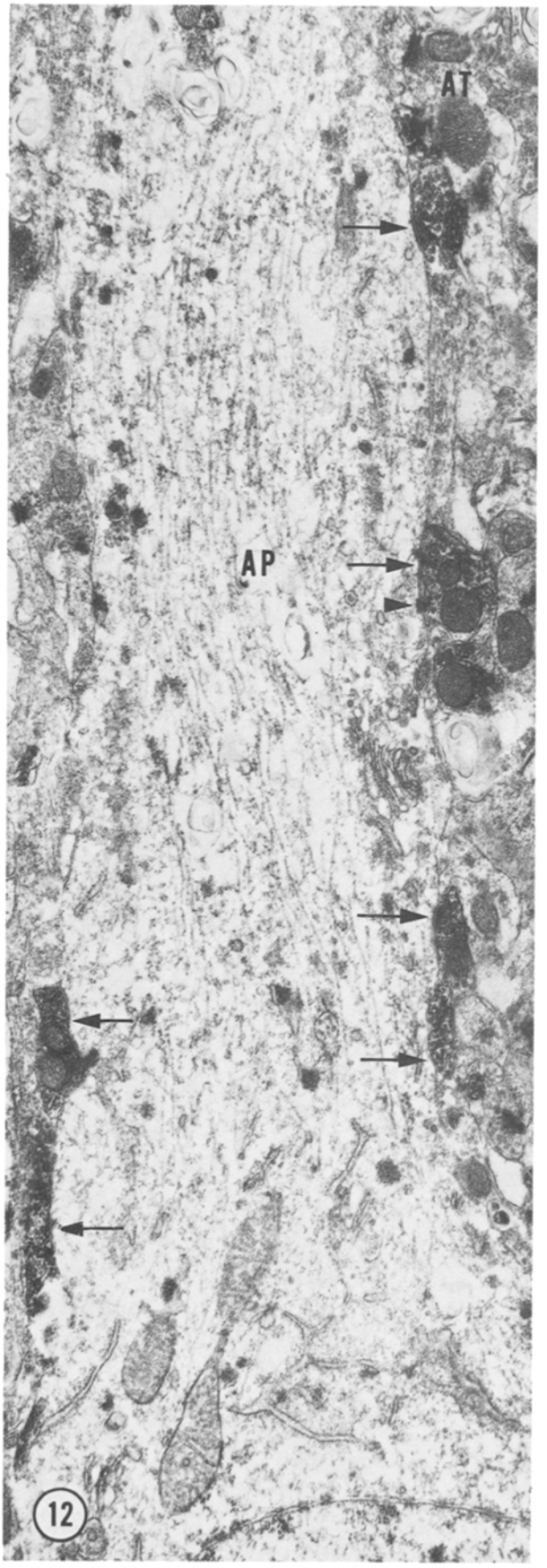
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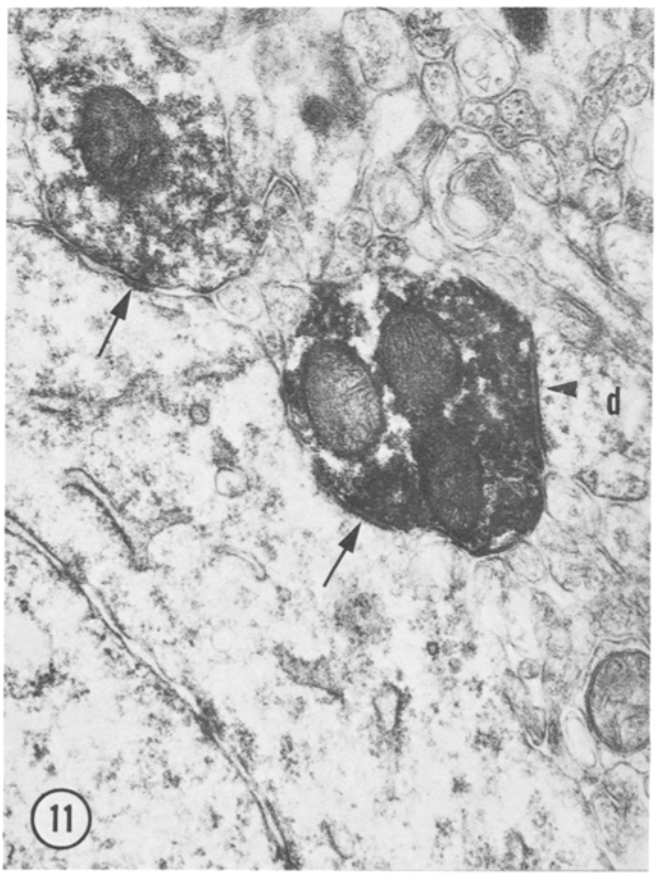
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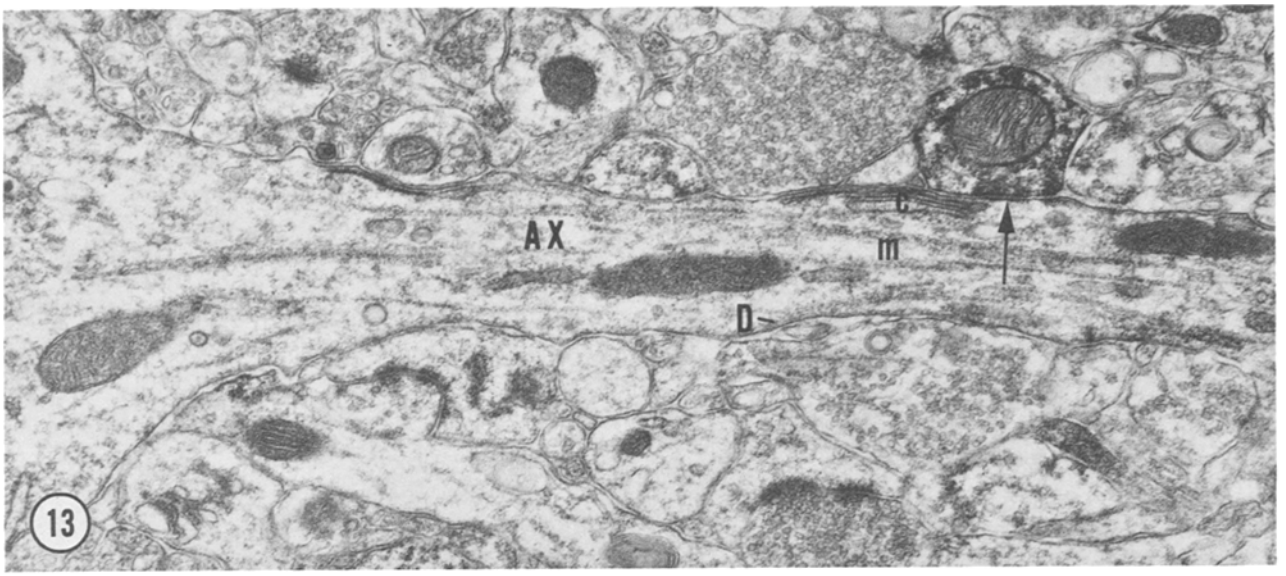

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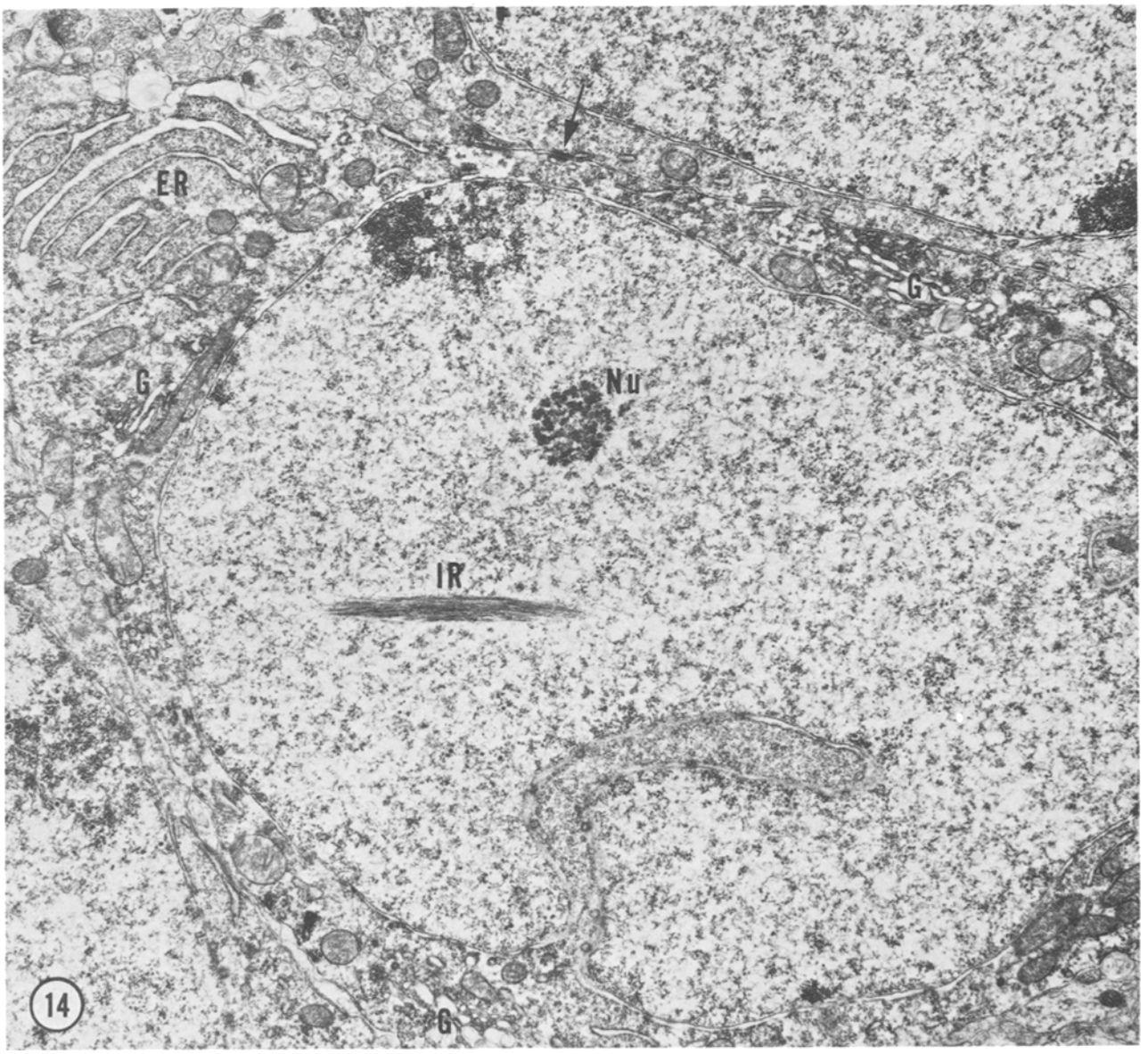

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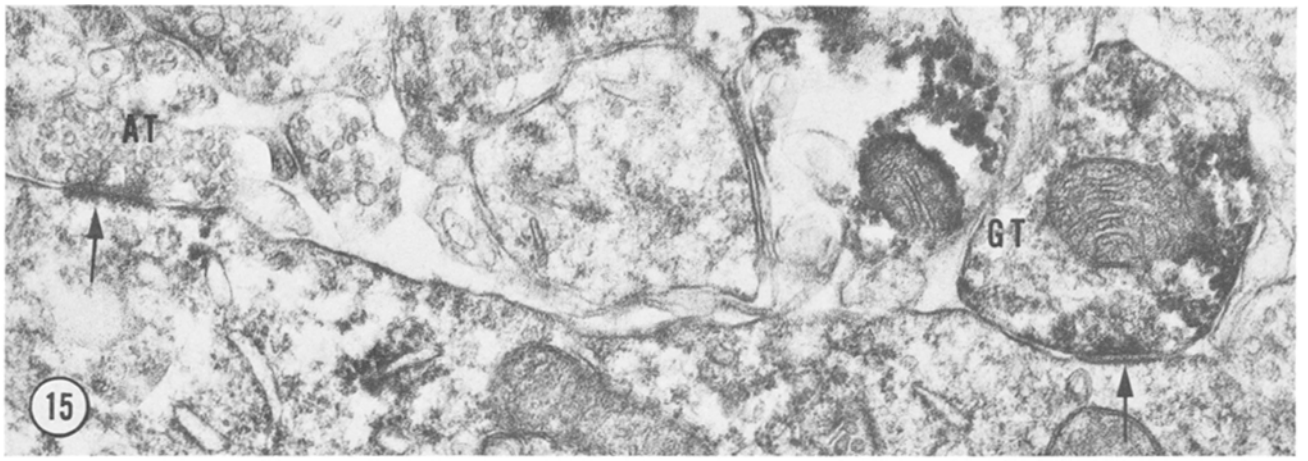
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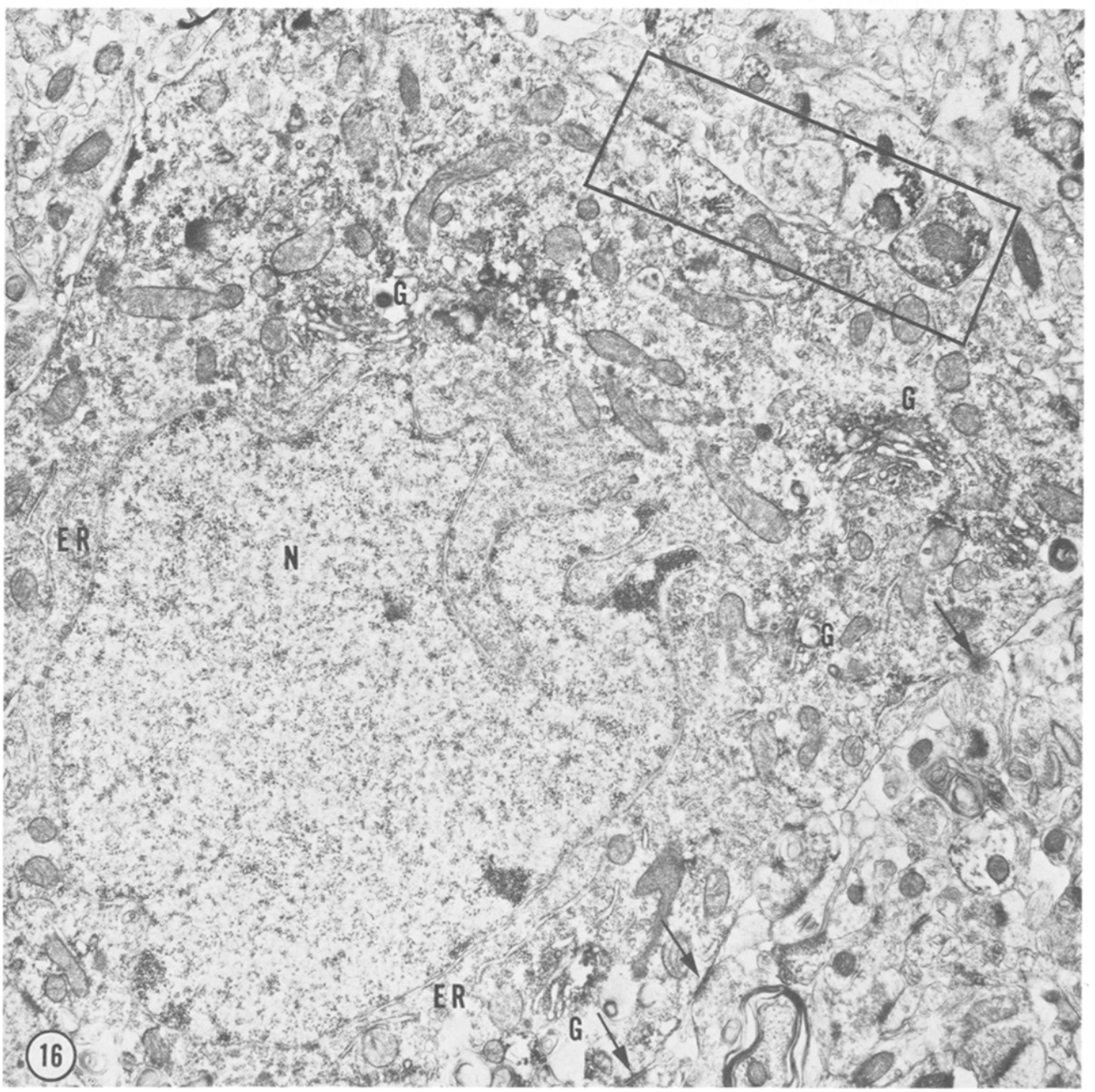
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above. The ultrastructural distribution of GAD-positive reaction product within axon terminals (Figs. 10, 11, 13, 15, and 17) occurred upon the surfaces of synaptic vesicles and mitochondria as described in previous studies (Wood *et al.*, 1976). GAD-positive axon terminals in the visual cortex all formed symmetric synaptic junctions, and the postsynaptic profiles were most commonly those of somata and dendritic shafts of pyramidal and stellate neurons (see Figs. 10, 11, 12 and 15). GAD-positive terminals were less frequently observed to form synapses with dendritic spines and with initial axonal segments of pyramidal neurons (Fig. 13). The axo-spinous synaptic relationships occurred in all cortical layers, and the GAD-positive axon terminals frequently shared postsynaptic spines with GAD-negative axon terminals which formed asymmetric synaptic junctions. Some of these postsynaptic spines were observed to arise from oblique, layer IV dendrites which were in continuity with the apical dendrites of pyramidal neurons.

The intracellular distribution of GAD-positive reaction product within somata and dendrites of cortical neurons was similar to that observed in other brain regions (Ribak *et al.*, 1977, 1978). The reaction product was most concentrated along the cisternae of the Golgi complex (Figs. 14, 16 and 18), and less concentrated upon the surfaces of mitochondria, microtubules and the cisternae of agranular endoplasmic reticulum. Reaction product was not observed within the nuclei of these GAD-positive somata nor was it observed adjacent to the cisternae of granular endoplasmic reticulum. Although trace amounts of reaction product were occasionally present adjacent to the plasma membranes of the GAD-positive somata and dendrites, the relative thicknesses of the postsynaptic densities associated with asymmetric and symmetric synapses were not obscured by this staining (Figs. 15–17). Thus, it was possible to identify a mixture of both asymmetric and symmetric synaptic junctions upon the somata and dendritic shafts of GAD-positive neurons (Fig. 15).

The nuclei of GAD-positive somata varied greatly in shape and size. Most nuclei had shapes which were generally round or oval, but when a soma abutted another soma or dendrite, its nucleus was somewhat flattened (Fig. 14). Some nuclei of GAD-positive somata were deeply infolded (Fig. 16), and most nuclei displayed

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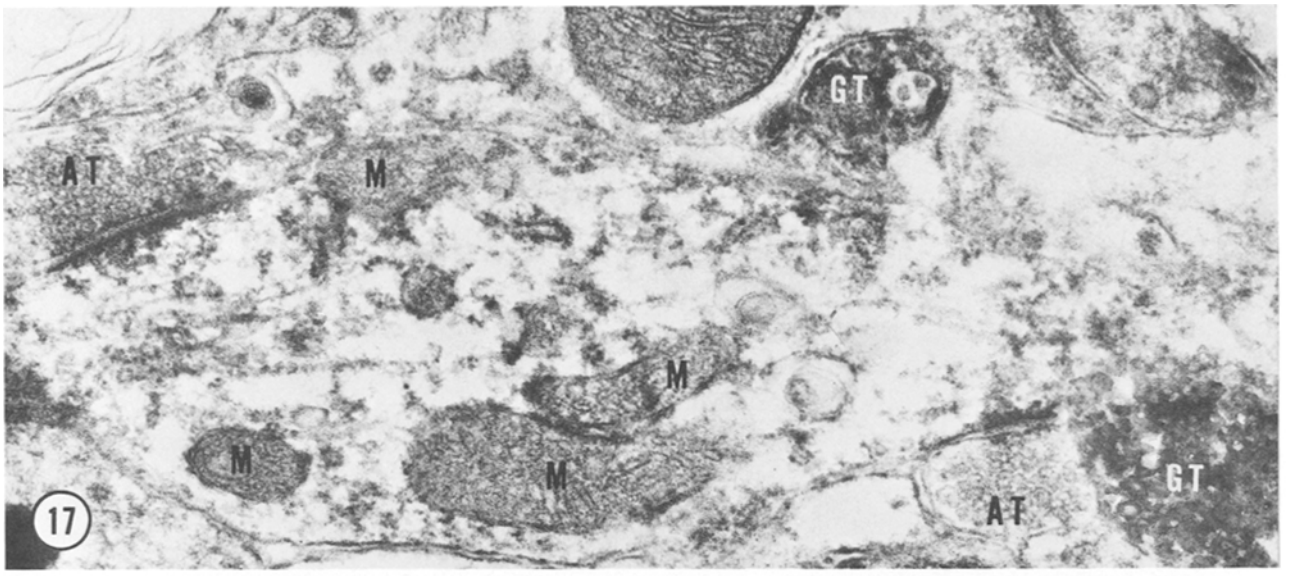
Figs. 17 and 18. Electron micrographs from colchicine-injected specimens of visual cortex incubated in anti-GAD serum. Fig. 17 shows a part of a dendrite which is continuous with a large GAD-positive soma (not shown). Some of the microtubules and mitochondria (M) appear to be sparsely decorated with GAD-positive reaction product. Two GAD-negative axon terminals (AT) form asymmetric synaptic junctions with this dendritic shaft, but the two GAD-positive terminals (GT) do not appear to form synaptic contacts in this micrograph. x 48 000. Fig. 18 shows a large GAD-positive soma from Layer III that contains only a grazed portion of its nucleus (N). Electron-opaque reaction product for GAD is concentrated around the cisternae of the Golgi complex (G). The cisternae of the granular endoplasmic reticulum (ER) lack reaction product and aggregate into small Nissl bodies. This soma forms symmetric synapses with many GAD-positive terminals (arrows). A somal spine (arrowhead) is present but is not readily apparent at this low magnification. This feature is also characteristic of sparsely-spinous and aspinous stellate neurons (see Peters and Fairén, 1977). x 9000.



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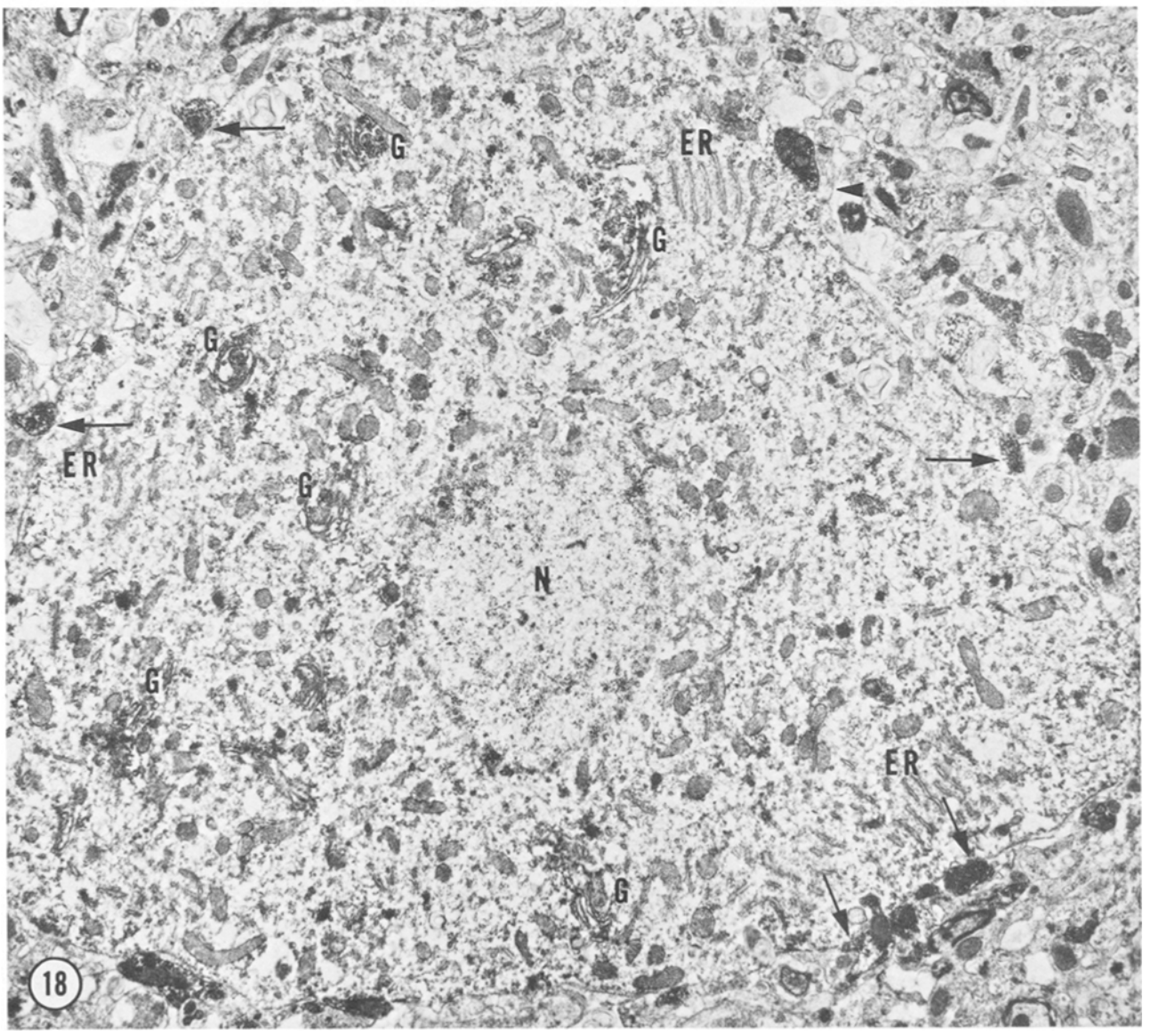

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some degree of irregularity in the contours of the nuclear envelopes. The chromatin within the nuclei of GAD-positive somata was rather evenly distributed except for small concentrations adjacent to the nuclear envelope (Figs. 14 and 16). Nucleoli and intranuclear rods or sheets were also present in random sections of GAD-positive somata (Fig. 14). Although intranuclear rods were not found in every GAD-positive soma observed in random sections, they were present in the two GAD-positive somata which were analysed in a series of 50 serial sections.

The rim of cytoplasm surrounding the nucleus of each GAD-positive soma varied considerably in its thickness and in its quantity of organelles. Small GAD-positive somata exhibited only thin rims of perikaryal cytoplasm which contained many mitochondria and a few scattered cisternae of the Golgi apparatus and the granular endoplasmic reticulum. The cisternae of both of these organelles were arranged parallel to the surface of the nuclear envelope in the small GAD-positive somata (Fig. 14). Cisternae of the granular endoplasmic reticulum were more abundant in large GAD-positive somata (Fig. 18) and in wider areas of the perikaryal cytoplasm of small somata (Fig. 14). The wider perikaryal cytoplasm of large GAD-positive somata also contained many parallel cisternae of the Golgi complex as well as an increase in the numbers of other organelles. In some of these large somata, the cisternae of the Golgi complex were clustered near the nuclei while the parallel cisternae of the granular endoplasmic reticulum were located near the somal perimeters (Fig. 18).

Dendrites which were continuous with GAD-positive somata were found to contain varying amounts of reaction product associated with the surfaces of microtubules and mitochondria. These dendrites were thin, varicose and tortuous processes which radiated from the somata in many different directions. Their shafts received symmetric synaptic contacts from GAD-positive axon terminals and asymmetric synaptic contacts from GAD-negative axon terminals (Fig. 17). The electron microscopic examination confirmed the light microscopic observations that reaction product was absent from the somata and dendrites of pyramidal neurons and was also absent from all neurons of visual cortex in sections incubated in control serum.

Discussion

The sizes, shapes, cytological characteristics and the laminar distributions of GAD-positive neurons are all similar to those characteristics of stellate neurons described in previous Golgi studies (Ramón y Cajal, 1911; Lorente de Nó, 1938; Marin-Padilla, 1969; Lund, 1973; Szentágothai, 1973; Jones, 1975; Feldman and Peters, 1978) and electron microscopic studies (Colonnier, 1968; Peters, 1971; Sloper, 1973; Szentágothai, 1973). However, the immunocytochemical method for GAD does not stain dendritic and axonal arborizations in their entirety (Ribak *et al.*, 1978) so that it is not possible to determine whether GAD is present in all of the subtypes of stellate neurons described in Golgi studies, i.e., Cajal-Retzius cells,

horizontal cells, Martinotti cells, basket cells and double bouquet dendritic cells or the nine varieties described by Jones, (1975). Some GAD-positive neurons appear to be horizontal cells as determined by their somal shape and proximal dendritic staining. Other GAD-positive neurons may be basket and double bouquet dendritic cells or stellate types 1–4 of Jones (1975) as these neuronal types all project axon terminals to the sites which correspond in location to those of GAD-positive terminals. The dendrites of these types of stellate neurons are known to be aspinous or sparsely-spinous from Golgi studies (see above references), and this is pertinent because GAD-positive neurons observed in the electron microscope have characteristics similar to those described by others for cortical aspinous and sparsely-spinous stellate neurons (LeVay, 1973; Fairén *et al.*, 1977; Parnavelas *et al.*, 1977; Somogyi, 1977; Peters and Fairén, 1978). For example, they have a mixture of asymmetric and symmetric synapses upon their somata and dendritic shafts, their dendrites are of small diameter and their axon terminals form symmetric synapses. Therefore, the present findings in combination with the anatomical evidence of other investigators indicates that GAD is present within aspinous and sparsely-spinous stellate neurons which have extensive, intracortical axonal arborizations.

In other brain regions, GAD-positive axon terminals form axo-somatic relationships which are similar to those described in this study for the pericellular baskets around pyramidal neurons. For example, in the cerebellar cortex, GAD-positive basket neurons form extensive axo-somatic synapses with Purkinje neurons (McLaughlin *et al.*, 1974; Ribak *et al.*, 1978), and in Ammon's horn, GAD-positive basket neurons form pericellular axonal plexuses around pyramidal and granule neurons (Barber and Saito, 1976; Ribak *et al.*, 1978). Physiological and pharmacological studies indicate that these basket synapses in the cerebellum and Ammon's horn are inhibitory and that this inhibition is mediated by GABA (Andersen *et al.*, 1964; Eccles *et al.*, 1967; Eccles, 1969). In each of these brain regions, the neuronal somata which receive numerous GABA-ergic axon terminals are those of projection neurons involved in establishing connections with other brain regions. This same basic relationship also exists in the visual cortex where the somata of pyramidal (i.e., projection) neurons receive numerous GAD-positive (GABA-ergic) axon terminals from aspinous stellate neurons. Thus a general rule appears to be that GABA-ergic, local circuit neurons establish extensive inhibitory synaptic plexuses with the somata and dendritic shafts of projection neurons.

A number of different studies of the visual cortex support the idea that some stellate neurons utilize GABA as an inhibitory neurotransmitter. For example, the results of physiological studies show that inhibitory mechanisms exist in the visual neocortex and suggest the presence of inhibitory short-axon neurons within layer IV (Watanabe *et al.*, 1966; Armstrong, 1968). Results from more recent studies using intracellular methods (Toyama *et al.*, 1974) suggest that inhibitory local circuit neurons are present in at least three cortical layers. One of these inhibitory neuronal types is present in layer IV and neurons of this class receive excitatory inputs from geniculocortical fibres. Another inhibitory type is present in layers II and VI, and

neurons of this class are excited disynaptically by the geniculocortical fibres. This latter type of inhibitory neuron may be excited by the axons of spinous stellate and pyramidal neurons since the neuroanatomical connections of the geniculocortical fibres and of the neurons innervated by them support this cortical circuitry (Lorente de Nó, 1938; LeVay, 1973; Jones, 1975; LeVay and Gilbert, 1976; Peters *et al.*, 1976; White, 1976). In addition, the results of pharmacological studies show that neocortical inhibition is mediated by GABA. The iontophoresis of GABA into the cerebral cortex has an inhibitory effect on all cortical units tested (Dreifuss *et al.*, 1969; Wallingford *et al.*, 1973) and this effect is blocked by the GABA antagonist, bicuculline (Curtis and Felix, 1971). In the cat visual cortex, the changes induced by bicuculline upon the recorded receptive fields of simple and complex cells indicates that the directional specificity of these units is dependent upon GABA-mediated inhibition (Sillito, 1975). The presence of GAD within aspinous and sparsely-spinous stellate neurons of all cortical layers together with the evidence presented above strongly suggests that these neurons effect GABA-mediated inhibition in the visual cortex.

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